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## Suppression of the Fenton Reaction by Ceriporic Acids Produced by a Selective Lignin-Degrading Fungus, *Ceriporiopsis subvermispora*

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Biodegradation of lignin by white rot fungi proceeds in concert with the activation of molecular oxygen and redox cycling of transition metals, with the aid of oxidative and reductive enzymes. When wood degrading fungi are colonized in woods, their extracellular enzymes are not able to diffuse into the intact wood cell walls because the enzymes are too large to penetrate the pores of the wood cell walls<sup>1</sup>. A cellulolytic active oxygen species, hydroxyl radical ( $\cdot\text{OH}$ ) is a principal low molecular mass oxidant that erodes wood cell walls to enhance the accessibility of the extracellular enzymes of wood rot fungi to wood cell wall components<sup>2</sup>. Hydroxyl radicals are produced by the reaction of  $\text{Fe}^{2+}$  with  $\text{H}_2\text{O}_2$  (Fenton reaction;  $\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{OH}^- + \cdot\text{OH}$ ), although involvement of other oxidizing species such as  $(\text{Fe}=\text{O})^{2+}$  have often been suggested. It is known that  $\text{Fe}^{3+}$  also reacts with  $\text{H}_2\text{O}_2$  to generate  $\text{Fe}^{2+}$  and  $\text{O}_2\cdot^-$ . This reaction is slower than the reaction of  $\text{H}_2\text{O}_2$  with  $\text{Fe}^{2+}$  at physiological pH and much depends on the ligand to the iron. In the Fenton system, catalysts for the reductive half cycle ( $\text{Fe}^{3+} \rightarrow \text{Fe}^{2+}$ ) accelerate the hydroxyl radical formation. Wood rot fungi have versatile enzymatic and non-enzymatic systems to accelerate the reductive half cycle. For instance, reduction of  $\text{Fe}^{3+}$  is catalyzed by  $\text{O}_2\cdot^-$  which is produced by the reduction of molecular oxygen with reductive radicals such as  $\text{CO}_2\cdot^-$  and semiquinone radicals from lignin fragments.  $\text{Fe}^{3+}$  is directly reduced by lignin-derived phenols such as guaiacol and catechol. Since the biodegradation of lignin produces free radicals and phenols in the presence of molecular oxygen and iron, oxidative degradation of cellulose by hydroxyl radicals is inevitable if some inhibition systems for the iron redox reactions are not involved during wood decay processes.

In contrast to brown rot and non-selective white rot fungi, selective lignin-degrading fungi like *Ceriporiopsis subvermispora* are able to decompose lignin in wood cell walls without the intensive damage of cellulose<sup>1</sup>. This indicates that the selective white rot fungus possesses unknown extracellular systems that attenuate the production of hydroxyl radicals. We herein report that ceriporic acid B, an alkylitaconate produced from *C. subvermispora* strongly suppressed the Fenton reactions even in the presence of the  $\text{Fe}^{3+}$  reductants such as

hydroquinone.

### Effect of ceriporic acid B on reduction of ferric ions

1-Nonadecene-2,3-dicarboxylic acid (ceriporic acid B) was synthesized using the Grignard reaction. The NMR and MS spectra of the synthetic compound were identical to those isolated from the cultures of *C. subvermispora* FP 90031<sup>3</sup>. Inhibitory effects of ceriporic acids on reduction of  $\text{Fe}^{3+}$  were examined by pre-mixing a solution (1,600  $\mu\text{l}$ ) of 0.02 mM  $\text{FeCl}_3$  and 0.3% Tween 20 in 20 mM sodium succinate buffer (pH 4.0) for 0.5 h at 25°C, with or without 0.02 mM ceriporic acid B. Next, 200  $\mu\text{l}$  of 3.0 mM disodium bathophenanthroline disulfonate (BPS) and 100  $\mu\text{l}$  of 40 mM reductant were added and absorption at 533 nm was monitored on a Hitachi U-2000A spectrophotometer (Tokyo Japan). As a reductant, freshly prepared solutions of ascorbic acid were used. The results obtained showed that reduction of  $\text{Fe}^{3+}$  ions by ascorbic acid was inhibited by the presence of ceriporic acid B in the same succinate buffer. The extent of inhibition for the reduction by ascorbic acid during the first 3 min reached 96%.

### Suppression of the Fenton reactions by ceriporic acid B

Hydroxyl radicals produced by the reaction of iron and  $\text{H}_2\text{O}_2$  were determined by the spin trapping of the secondary radicals from ethanol<sup>4</sup>. ESR spectral recordings were made in a flat cell on a JEOL FR-30 X-band ESR spectrometer operating at room temperature with a modulation amplitude of 0.079 mT, a time constant of 0.10 sec, a scanning time of 2 min and a microwave power of 4 mW. Recently, we reported inhibition of  $\cdot\text{OH}$  production by ceriporic acid B in aqueous media containing no buffer salts. In the present study, the effects of ceriporic acid B on the  $\cdot\text{OH}$  production was quantified in sodium succinate buffer (pH 4.0). When  $\text{H}_2\text{O}_2$  (0.5 mM) was added to a solution (200  $\mu\text{l}$ ) containing  $\text{Fe}^{3+}$  (0.1 mM), ethanol (3.42 M), hydroquinone (0.05 mM) and 4-POBN (100 mM), a spin adduct of the  $\alpha$ -hydroxyethyl radical was produced. However, in the presence of 1-nonadecene-2,3-dicarboxylic acid (ceriporic acid B, 3 mM), the production of  $\cdot\text{OH}$  was strongly inhibited as observed in the reactions without the succinate buffer<sup>5</sup>. In reactions of  $\text{Fe}^{3+}$  with  $\text{H}_2\text{O}_2$  with and without hydroquinone, inhibition of  $\cdot\text{OH}$  production

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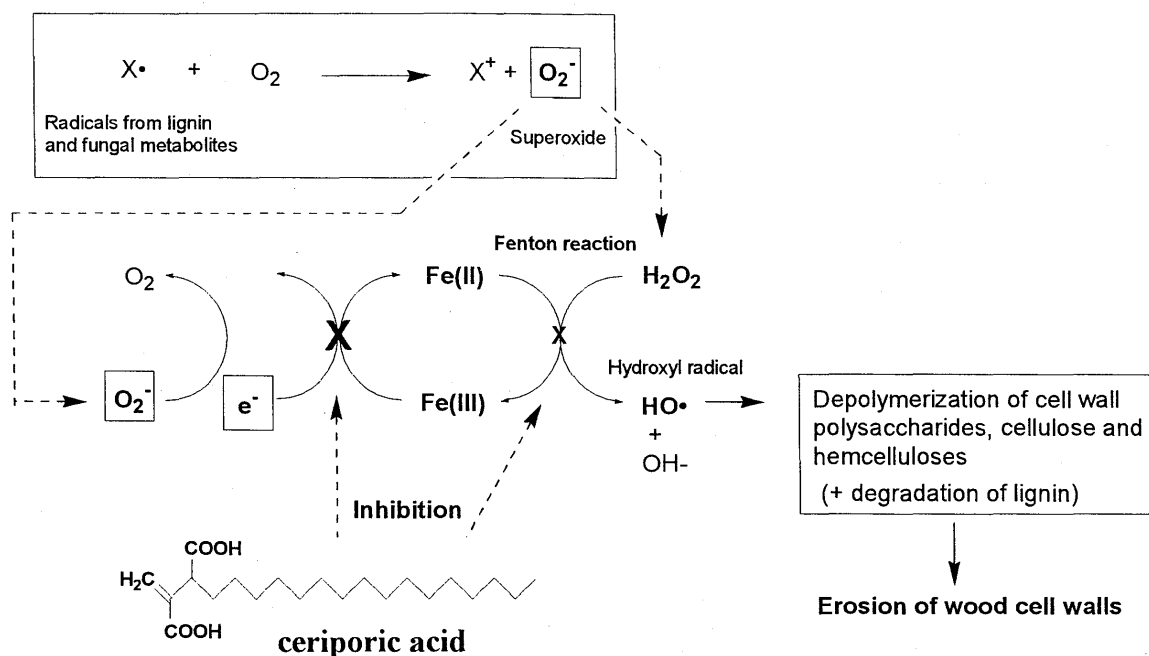


Fig. 1. Acceleration and inhibition of the production of hydroxyl radicals by wood rot fungi. In selective white rot, lignin is degraded without penetration of the extracellular enzymes into wood cell wall regions. Ceriporic acids, metabolites of *C. subvermispora* inhibit the iron redox reactions to suppress the formation of  $\cdot OH$ .

by ceriporic acid B after 15 min in the buffer reached 92 and 94%, respectively. In direct reactions of  $Fe^{2+}$  with  $H_2O_2$ , inhibition of the  $\cdot OH$  production by ceriporic acid B during the first 1 min was 56%. However,  $\cdot OH$  was not produced after 1 min. This is in accordance with the experiments without the buffer<sup>5)</sup>. The marked differences in the inhibition rate before and after 1 min suggest that inhibitory effects of ceriporic acid B on the  $\cdot OH$  production is ascribed to the reductive half cycle from  $Fe^{3+}$  to  $Fe^{2+}$ . The inhibition was not observed when dimethyl ester of the alkylitaconic acid was used instead of the free dicarboxylic acid. Thus, ceriporic acids suppressed the Fenton reaction by ion-redox interactions at weak acidic media suitable for the growth of this fungus on wood meal cultures. Differences in reactivity with and without succinate buffer were found in the reactions of itaconic acid. Without succinate buffer, itaconic acid promoted the hydroxyl radical production. However, in succinate buffer, the rate of  $\cdot OH$  production in the presence of itaconate was the same as that of the control experiments

without the chelator compound.

In summary, ceriporic acids strongly attenuated the Fenton reaction by suppressing iron redox reactions in a weak acidic medium. Studies on ceriporic acids will lead to further understandings of the selective white rot by *C. subvermispora*.

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